

# There Must Be a Prokaryote Somewhere: Microbiology's Search for Itself†

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## INTRODUCTION

They cried, "Microbiology is dead! Long live microbiology!"

Long ago Darwin had a vision: "The time will come," he said, "... though I shall not live to see it, when we shall have very fairly true genealogical trees of each great kingdom of nature" (5). It has taken over a century of scientific progress to realize Darwin's prophecy. We are fortunate to be biologists at this time—indeed, doubly fortunate—for not only can "fairly true genealogical trees" now be constructed for *all* the great kingdoms but we can go beyond that, to something perhaps even Darwin dared not dream: the synthesis of the great kingdoms into a universal tree of life (33, 36).

This universal genealogical tree (Fig. 1) represents a significant departure from what scientists thought were the natural relationships among organisms even a decade or less ago and from what most high school and many college students are *still* taught. But its implications go far beyond genealogical relationships—to the very heart of the way we think about biology. And it is microbiologists in particular who should listen to the trees, as it were, for their discipline stands to be the most transformed, most rejuvenated, by what has been learned.

How many of you were taught that there are five kingdoms of organisms; the Animals, Plants, Fungi, Protists, and Monera? Note the position of the animal, plant, and fungal lineages in Fig. 1. Now, contrast these three collectively to the phylogenetic branchings encompassed by the Protists and all, in turn, to the panorama of lineages covered by Monera (the prokaryotes). By no stretch of the imagination are these five "kingdoms" of equivalent taxonomic rank. This five-kingdom picture (32)—which is central to the teaching of biology in most high schools today—is a distorted and counterproductive image of the genealogy of the living world.

How many of you were taught that all life could be accommodated in two superkingdoms, eukaryotes and prokaryotes? Again the formulation proves phylogenetically wanting (Fig. 1). However, in this case the effects extend far beyond the teaching of high school biology, for eukaryote/prokaryote has been a "central dogma" of biology for at least the last three decades, and all of us, knowingly or unknowingly, have been strongly influenced by it—just how strongly you will see as this lecture progresses.

The natural order of the living world, however, is readily

apparent from the topology of the universal tree: all life falls into one of three primary groupings, formally called domains (36)—the *Bacteria* (or eubacteria), the *Archaea* (formerly archaeobacteria), and the *Eucarya* (or eukaryotes). Although the *Bacteria* and the *Archaea* are both prokaryotic in cell type (whatever that now means), the members within each domain share many common molecular characteristics, making each of the three as distinct an entity for the biologist (33, 34), as elephants, ants, and flowers are for the layman. The universal genealogical tree (Fig. 1) also shows the *Archaea* to be a specific relative of the *Eucarya*—that I'll save for later.

I am not here, however, to sell you on the new three-domain concept, to dazzle you with the adventures of archae hunters (who do actually risk their lives now and then), to argue the ins and outs of phylogenetic analysis, or to talk about my favorite preoccupation, rapidly evolving lineages. My concern now is with the forest, not the trees. The developments that have been happening in biology over the last few decades are transforming the nature of that science in concept and in (institutional) structure. We must prepare ourselves for what lies ahead; i.e., we must gain a sufficiently broad overview that we can and will facilitate and shape this transformation.

What I specifically mean by this rather grandiose pronouncement is this: molecular/biochemical studies over the past several decades have increasingly involved molecular sequencing. The biologist cannot become immersed in the world of sequences without automatically focusing in an evolutionary direction (37). Almost all aspects of biology will now take on an evolutionary flavor, which in some cases will grow to become their essence. Not only will this "new" outlook reorganize every biological discipline, but also it will drastically alter their relationships to one another, which, of course, means changing our institutional structures as well as our outlook. And it is the science of microbiology, the most primitive of biological disciplines in an evolutionary sense, that will be the most affected and will become the most central to this reorganization. We need to know the hows and the whys of this process. Now is definitely not a time to go about interpreting the large and growing collection of new facts in old ways (or within outdated scientific structures); for what is at stake is the future of our science (and all that implies).

You may have wondered why I began this lecture saying "Microbiology is dead!". Perhaps now you begin to understand. That proclamation is the epitome of biology's problem today. Look about you; note what has happened to microbiology over the last several decades. Microbiology departments are disappearing. They were already tending to become de facto molecular biology/biochemistry departments in the

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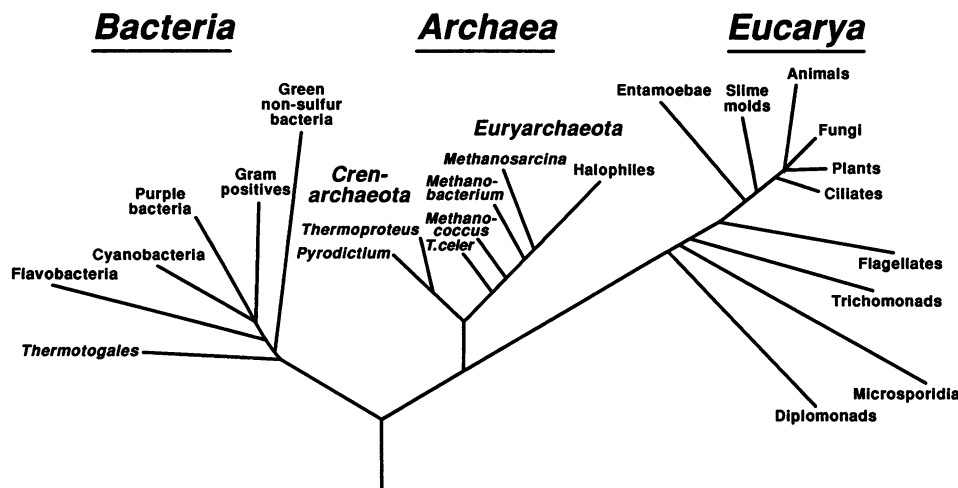


FIG. 1. Universal phylogenetic tree in rooted form, showing the three domains, *Archaea*, *Bacteria*, and *Eucarya*; adapted from the corresponding tree in reference 36, by the inclusion of more recent results concerning eukaryote phylogeny (22). The position of the root was determined by using the paralogous gene couple, translation elongation factors EFTu and EFG (13).

1960s. Now they are being eliminated, usually downgraded into ineffectual "microbiology programs" within larger departments of Molecular and Cellular Whatever. Microbiology is inadequately represented in the funding agencies. Students (and professors) genuinely interested in microbiology are increasingly hard to find. Microbiologists are afraid to teach real microbiology any more: the title of J. C. Ensign's lecture when he was given the 1992 Carski Foundation Distinguished Teaching Award says it all: "A Place for Bacterial Diversity in the Microbiology Curriculum: A Plea for an Endangered Species." Obviously, conventional scientific wisdom does not perceive microbiology, the study of microorganisms, as a vital, worthwhile discipline in its own right. They would have us believe that the future lies on the molecular level, with eukaryotic cell biology, with biotechnology, with the human genome initiative, and that microbiology's time is past. Its only raison d'être is to provide useful and easy-to-manipulate systems for biochemical, molecular, and genetic studies and to deal with certain medical, agricultural, and environmental problems. If microbiology isn't dead today, it definitely is moribund.

Now let us look at things from another perspective—the real world. Note again in Fig. 1 the portion of the universal tree that the microbial lineages encompass. That tree is saying to us in no uncertain terms "Long live Microbiology!". In other words, the sorry condition of that discipline today is the result of our distorted perceptions, perceptions not in tune with the position of microorganisms in the natural order of things. Why doesn't the study of living systems even *begin* to reflect the natural order?

This out-of-kilter situation came about largely for two reasons. One is microbiology's weakness, its stunted development, and the reaction this engendered in other biological disciplines (and administrative units). The other is a flaw in the molecular world view, its "dark side," if you will. Microbiology's weakness lies in its failure to develop its evolutionary (historical) dimension; molecular biology's flaw is in not realizing the importance of that dimension. (Molecular biology [and biochemistry] concerns itself with how something works in molecular mechanistic terms. How it came to be that way is unimportant, of no more interest scientifically than the succession of Egyptian pharaohs. In the molecular perspective *Escherichia coli* arose through a series of historical accidents that

have no bearing on our understanding of *E. coli*!) Molecular biology fails to recognize a basic Darwinian principle, namely, that an organism's evolutionary history is part and parcel of its nature and that the organism (or its component parts, for that matter) is not intelligible apart from that history. The fact that microbiology is at base an evolutionary discipline has (at least in recent times) never been appreciated and the fact that during its development microbiology could not become an evolutionarily based discipline lead to the distorted, entirely too superficial concept of microbiology we now have.

I need to convince you of the importance of changing our current outlook on biology, microbiology in particular. There's a lot riding on it. As I said, biology is on the doorstep of a new era, a resurgence of evolutionary study, and we microbiologists have to lead the way.

To understand where microbiology is going, we have to understand where it came from; we need to concern ourselves in particular with the history of microbial phylogeny, the principal determinant in microbiology's development. There were two important phases or periods in this process. In the earlier Darwinian or Classical Period—so-called because of its broad (though primitive) perspective—microbiologists had a fundamental concern with microbial diversity and the natural relationships among microorganisms. But as molecular biology (and biochemistry) gained ascendancy, microbiology slipped (unnoticed) into a Dark Age—during which, despite great progress in certain areas, the intellectual heritage of the Classical Period was lost and concern with microorganisms as individuals (individual species) gave way to a preoccupation with molecules and biochemical pathways. A third Period is now on the horizon. The technical power developed in the Dark Age remains (and continues to grow); but microbiology's outlook is returning to that of the Classical Period—making this forthcoming period a true Renaissance.

Some 15 years ago, when the discovery of the *Archaea* was first reported (9, 35), microbiologists and biologists in general reacted in what can only be called an irrational way: the discovery was generally rejected out of hand, even derisively dismissed. However, this is fully understandable; the discovery of a "third form of life" (as the *Archaea* was originally called) had breached a paradigm—and the good citizens were taking up arms to repel the invaders. The problem for me and my collaborators at that time (and since) became that of convinc-

ing biologists (especially microbiologists) that their outlook was flawed and needed to change. This is never easy, however; recalcitrance, bitterness, and a lot of vested interests are involved. The fact that I and my cohorts have been at this task for well over a decade now, with only partial success, shows you how difficult changing a paradigm can be.

You should know several things about scientific paradigms, those frameworks within which scientific disciplines develop and operate. For one, a paradigm comprises more than the scientific know-how and knowledge of its discipline; it also embodies the field's collective value structure, prejudices, and lore. For another, a paradigm is extremely hegemonic and strenuously resists change. And, finally, by its nature a paradigm is a somewhat arbitrary, unpredictable construct. What this means in the present context is that microbiology today need not necessarily have been what it is, but what it is does not want to change. Facts alone do little to help in such a situation; a perspective, an overview from which the tenets implicit in the paradigm become evident, is needed. There is no better way to gain such a perspective than through historical analysis of the discipline's development.

Thus it was, in my attempt to develop the necessary historical perspective, that I first encountered the early "forgotten" writings of Roger Stanier and C. B. van Niel. These two biologists were the only consistently insightful and articulate reporters of the early search for a microbial phylogeny, the "natural relationships" as the problem was then commonly known. For that reason I will introduce you to microbiology's Classical Period mainly through the words of these two men. But mark you, there will be two C. B. van Niels and two Roger Staniers, for their careers spanned and reflect both sides of the watershed separating the Classical Period from the ensuing Dark Age.

### THE CLASSICAL PERIOD

Although microorganisms were discovered by Leeuwenhoek in 1675, they didn't come in for meaningful scientific study for another two centuries. In Darwin's time the problem was merely to develop, no matter how primitive, some concept of a microorganism. Haeckel offered what at the time was a revolutionary suggestion: microorganisms were neither plants nor animals; they were a kingdom unto themselves he called Protista. Along with various microscopic eukaryotes the new kingdom included the bacteria, or "Moneres," as one of its high-level groupings. Unfortunately, Haeckel did not include the blue-green algae among the Moneres; they remained Plants (with which they had traditionally been associated for obvious reasons). However, because of this, Ferdinand Cohn, the great German microbiologist, did not accept Haeckel's new classification. For him the uniqueness of the bacteria was overriding. Although he retained the old view that *all* bacteria are plants, Cohn fully appreciated the profound distinction between bacteria and all higher forms and, like most of his contemporaries, saw the former as very primitive. In his words (6) (translated): "[Bacteria constitute the] first and simplest division of living organisms, that to me seems naturally differentiated from the higher plant groups, even though their characteristic attributes are more of a negative than a positive nature."

Although I have yet to find a direct quote to this effect—the point undoubtedly being self-evident—microbiologists of the Classical Period appreciated the basic Darwinian principle stated above; it underlay and spurred their attempts during that time to determine the natural bacterial relationships. Writing in the 1930s, Kluyver and van Niel (15) said:

... the only truly scientific foundation of classification is to be found in appreciation of the available facts from a phylogenetic point of view. Only in this way can the natural interrelationships of the various bacteria be properly understood. ... it cannot be denied that the studies in comparative morphology made by botanists and zoologists have made phylogeny a reality. Under these circumstances it seems appropriate to accept the phylogenetic principle also in bacteriological classification. ...

A true reconstruction of the course of evolution is the ideal of every taxonomist.

From here Kluyver and van Niel went on to produce what they believed was a valid approximation to a natural bacterial classification, recognizing that the course of bacterial evolution is in no sense directly knowable, and, therefore, that they might be wrong.

van Niel subsequently continued his work on a natural bacterial system with his former student Stanier; in their writings you can sense the importance that was attached to determining these relationships during the Classical Period. This was no passing fancy: "... the mere fact that a particular phylogenetic scheme has been shown to be unsound by later work is not a valid reason for total rejection of the phylogenetic approach" (26).

I will not detail the natural classifications produced during this early period, for very few of the proposed higher taxa were valid. However, we *can* learn from the general principles upon which these systems were based, from the attitudes microbiologists took toward them, and from the reasons these systems failed.

The principles used were simple and borrowed from zoological classification: morphological characters are primary; the morphologically simplest forms are the ancestral ones; and these evolved, progressively developed, into more complicated, intricate forms—"levels of improvement" as Julian Huxley called them (12). In the words of these early microbiologists (15),

There is no doubt that ... morphology remains the first and most reliable guide. ... the indispensability of physiological characters [in] classification has [also] been generally accepted, which is only natural because, after all, these physiological differences [are] expressions of variations in submicroscopical morphology.

... the diversity of bacterial forms is the outcome of various independent morphological evolutions which have had their starting-point in the simplest form both existent and conceivable: the sphere.

The concept of evolutionary development was frequently invoked by microbiologists of this period [*italics added*]:

The *highest developmental stage* in the group of spherical organisms is in all probability displayed by the cocci able to form endospores ... (15)

... endospore forming rods with peritrichous flagella represent a *higher stage of development* in these groups ... (15)

... *further development* of these universally immotile bacteria can have given rise to the mycobacteria which apparently form the connecting link with the simpler actinomycetes ... (15)

... the myxobacteria have [likely] *developed through forms* ... in which the fruiting stage is absent ... [*i.e.*,] the cytophagas ... (26).

These early efforts at constructing a natural bacterial system did not succeed for reasons completely beyond microbiologists' control: the bacterial properties upon which their systems were necessarily based—gross cellular morphology, motility, general physiological characters, colony characteristics, and so on—were just too simple, variable, or otherwise uninterpretable to be phylogenetically reliable. Classical microbiologists were quite aware of this possibility, but they couldn't have known how severe the problem was.

As understanding of bacteria increased, so too did the realization that the search for a natural microbial system had been a Sisyphean labor. In his Cold Spring Harbor address of 1946, van Niel (29) delivered a sober assessment:

[In] plant and animal systematics . . . morphological characters are intricately linked with the rather elaborate life histories of the organisms. But this does not apply to the bacteria . . . [where] morphological criteria have, on the whole, a far more restricted significance, and do not justify any conclusions about phylogeny. . . [Nor can we] yet use physiological or biochemical characters as a sound guide for the development of a 'natural system' of classification of the bacteria.

van Niel's 1946 address brings us to the watershed. From his phrasing it is clear that he still operated from the Classical perspective, which implicitly understood that bacterial evolutionary histories are essential to understanding what bacteria are, and explicitly recognized the importance of a natural bacterial system and the need to persist in the pursuit of one. Indeed, he was quick to follow the above statement with, "However, . . . the search for a basis upon which a 'natural system' can be constructed must continue." (29).

### THE DARK AGE

Within a decade, however, van Niel's outlook appeared to change, for he wrote as follows in 1955 (30):

What made Winogradsky (1952) grant that the systematics of plants and animals on the basis of the Linnean system is defensible, while contending that a similar classification of bacteria is out of the question? The answer must be obvious to those who recognize . . . that comparable efforts in the realm of the bacteria (and bluegreen algae) are doomed to failure because it does not appear likely that criteria of truly phylogenetic significance can be devised for these organisms.

The meaning of this change in tone becomes clearer when van Niel later joins Roger Stanier to say (27):

Any good biologist finds it intellectually distressing to devote his life to the study of a group that cannot be readily and satisfactorily defined in biological terms; and the abiding intellectual scandal of bacteriology has been the absence of a clear concept of a bacterium.

Our first joint attempt to deal with this problem was made 20 years ago (Stanier and van Niel, 1941). At the time, our answer was framed in an elaborate taxonomic proposal, which neither of us cares any longer to defend. . . we have become sceptical about the value of developing formal taxonomic systems for bacteria . . .

In these two quotes you can sense a new outlook—a new attitude toward the problem of the natural relationships,

toward the possibility of determining them, and perhaps even toward the value in doing so. In any case, there can be little doubt that these two microbiologists and others now believed that the search for bacterial evolutionary relationships is a futile, worthless effort. This assessment was echoed and embellished in successive editions of *The Microbial World*, arguably the greatest, and certainly the most influential, microbiology text ever written:

. . . any systematic attempt to construct a detailed scheme of natural relationships becomes the purest speculation . . . the ultimate scientific goal of biological classification cannot be achieved in the case of bacteria. (24).

. . . most modern [bacterial] taxonomists have explicitly abandoned the phylogenetic approach . . . (25).

I see the problem here as follows. Conceptually the Classical perspective was correct as regards the importance of determining microbial relationships: they *are* the key to a "concept of a bacterium." Technically, however, the Classical paradigm was so weak that these relationships could not be determined. Where technology was beginning to push microbiology was in the study of cellular ultrastructure, biochemical pathways, and molecular and genetic mechanisms—areas that were progress laden (but at that time yielded little in the way of evolutionary information). It was only natural that microbiologists would turn to these approaches to learn what they could about bacteria. What they didn't appreciate was that in doing so they were accepting the molecular world view and, with it, a disdain for evolutionary matters (and for microorganisms *per se*). From here on it was molecules and mechanisms, not organisms and relationships.

As a consequence of their new, acquired outlook, microbiologists had to redefine what they meant by a "concept of a bacterium." This they apparently thought they could do through resurrecting the prokaryote-eukaryote dichotomy:

. . . among organisms there are two different organizational patterns of cells, which Chatton (1937) called, with singular prescience, the eukaryotic and prokaryotic type. *The distinctive property of bacteria and blue-green algae is the prokaryotic nature of their cells.* . . .

[It is] the essential differences between these two cell types, upon which rests our only hope of more clearly formulating a 'concept of a bacterium'. (27).

Microbiologists of the Classical Period had distinguished between prokaryotes and eukaryotes, although rarely using those names—you can see it in the previous quote from Ferdinand Cohn, for example. However, before they became "enlightened," microbiologists found little merit in the distinction, for in its original cytological framing the definition of the prokaryote was purely negative—a cell not possessing this or that eukaryotic cellular feature. The "taxon" prokaryote so defined was possibly polyphyletic and certainly phylogenetically meaningless. To quote Pringsheim (and recall Cohn): "The entirely negative characteristics upon which [the prokaryote] is based should be noted, and the possibility of . . . convergent evolution . . . be seriously considered" (17). Stanier and van Niel at one point had themselves even said, ". . . we believe that the three major groups among the bacteria [Eubacteriae, Myxobacteriae, and Spirochaetae] are of polyphyletic origin" (26).

Frankly, I think that by the 1950s van Niel and Stanier had become disenchanted with their fruitless search for bacterial relationships and had written the whole thing off and that

neither one wished to put further effort or serious thought into the problem. If so, this is understandable; I have done the same thing with regard to the genetic code. Unfortunately, almost all microbiologists now thought that way; this was so easy to do in the brave new molecular world, where evolutionary relationships counted for naught.

Thus, when the prokaryote and eukaryote came to be redefined in positive and comparable (molecular) terms, a dreadful mistake was made: the intellectual heritage of the Classical Period was forgotten. It became an a priori assumption for microbiologists and biologists alike that "prokaryote" was a monophyletic taxon. Why not? Eukaryote was obviously so. As a result there were no serious objections raised to (re)defining the prokaryote in molecular terms on the basis of one, or at best a few, "representative" species, principally *Escherichia coli*. As a result, all biologists came firmly to accept that in a genealogical sense there were two basic types of living systems on Earth. Indeed, at one time I too believed in the eukaryote/prokaryote dogma, as strongly as the next person.

How did the "concept of a bacterium" fare under this new regime? The great accomplishment of this Dark Age was to define the prokaryote in positive terms, by using molecules and functions for which there were homologs among eukaryotes; the old negative definition of the prokaryote was now obsolete. But remember, the new definition of "prokaryote" rested solely on *E. coli* and its cousins. The molecular distinction between prokaryote and eukaryote reached deep into the heart of the cell, in terms of chromosomal organizations, control mechanisms, ribosome structures, and so on. However, our "concept of a eukaryote" does not turn on these molecular distinctions; it remains based upon the understanding implicit in eukaryote phylogeny. Why, then, should the concept of a prokaryote be any different? The historical (evolutionary) dimension is essential to understanding *all* living systems, and the molecular distinctions between prokaryote and eukaryote do not provide that.

Some of you may believe my criticism of the microbiology of the 1950s and 1960s to be unfair, to be a mean-spirited and/or pointless exercise in hindsight. If so, I must disagree: microbiology of that era had clearly undergone a paradigm shift that caused it to discount and deliberately close its eyes to the problem of bacterial relationships; and this must be fully appreciated. By the time that van Niel in 1955 (following Winogradsky's 1952 lead) concluded that "criteria of truly phylogenetic significance can[not] be devised for these organisms" (30), Sanger had already sequenced insulin; and by the early 1960s fibrinopeptide, hemoglobin, and cytochrome *c* sequences were being used to verify, refine, or extend eukaryotic phylogenies. Thus, by 1965, when Zuckerkandl and Pauling published their influential article, *Molecules as Documents of Evolutionary History* (37), the enormous potential for inferring genealogical relationships inherent in molecular sequences was there for all to see. Here was the historical dimension that microbiologists earlier had so ardently sought. Here were Kluver and van Niel's "variations in submicroscopical morphology" that underlay microbial physiology (15). And, best of all, here obviously were attributes precise and complex enough that the bugbear of microbial classification, i.e., characteristics too simple and ill defined to be phylogenetically reliable, was gone.

Yet few microbiologists sought to apply any of the new molecular technologies to the problem of microbial relationships, and then only in restricted and uninspired ways: to weed out "misclassified" species, for example. The response by microbiologists in general to even these accomplishments was nothing short of phlegmatic. No microbiologist thought to use

actual molecular sequences seriously for phylogenetic purposes. (There is an interesting exception here. Cytochrome *c* sequences were actually used to investigate relationships among what are now called the proteobacteria. The most interesting aspect of this work, however, is that when the study was brought to its culmination, in a 1979 issue of *Nature*, the authors found their phylogeny to be at variance with *Bergey's Manual* and, in their words, concluded "only God can make a tree" [1, 2]). The old dream of a grand global microbial system—make no mistake about it—was gone. *This* is why the molecular sequence revolution in microbial systematics came about for reasons having nothing to do with microbiology's glorious past vision; and *this* is why that revolution was initiated by someone not having a microbiologist's perspective.

Which brings us to 1977—when the archaea appeared suddenly on the scene. Two aspects of this discovery require explanation: (i) why was it so unexpected, so sharp, so precipitous—when most major biological discoveries tend to be foreshadowed by experimental hints, and/or some conceptual/theoretical expectation; and (ii) what accounts for the reaction of the biological community?

It turns out that well before 1977 there existed a *prima facie* case for something being strange about this monolithic world of the prokaryote, but no one recognized it. Ether-linked lipids with branched chains, i.e., archaeal lipids, had been known in the extreme halophiles since the early 1960s at least (21), and they were later also found in *Sulfolobus* and *Thermoplasma* species. Did this suggest a new and very different grouping to anyone? No, not at all. In the words of one of those involved in these studies (4), "The fact that *Sulfolobus* and *Thermoplasma* have similar lipids is . . . almost certainly . . . explained by convergent evolution. This hypothesis is strengthened by the fact that *Halobacterium*, another quite different organism, also has lipids similar to those of the two acidophilic thermophiles." This is a statement typical of the times, and statements of this sort make little or no sense unless you understand two things: (i) the notion of polyphyly among prokaryotes was unthinkable in that era, and (ii) microbiologists then viewed all organisms growing in "extreme" niches as having *adapted* to those niches. If such an organism exhibited some unusual biochemical feature, then that "unusual" feature was the result of adaptation to the organism's "unusual" niche. But other indications were there as well, such as the methanogen cell walls lacking peptidoglycan (14) and the amazing assortment of novel coenzymes these organisms possessed. Yet these things weren't enough to shake the faith: a prokaryote is a prokaryote is a prokaryote.

It was this same faith, as you can now appreciate, that accounted for the negative reaction to the discovery of the archaea. Most of this reaction did not appear in print, which is regrettable, for it would make interesting and instructive reading today. However, this again is the way of paradigms: when facts or concepts arise that challenge them, these tend to be ignored. If that is not possible, they are scoffed at and otherwise communally rejected—*informally*. (And the archaea were greeted with reactions aplenty of this type.) Only as a last resort will the paradigm *formally* contest the novelty that threatens it (i.e., treat it scientifically).

Thanks to the prokaryote-eukaryote dogma, the archaeal concept got off to a rather rocky start. Yet, I cannot imagine that any microbiologist in the Classical Period, especially the early C. B. van Niel and the young Roger Stanier, would have greeted the archaea with anything short of delight, for their discovery signaled that the natural relationships among the prokaryotes could finally be reliably determined. And, as a previous quote showed, it wouldn't have surprised these two

biologists at all if prokaryotes turned out to be polyphyletic. Now you see what a flawed paradigm can do. That midcentury shift in microbiology's world view—dismissing the search for microbial relationships, embracing the prokaryote-eukaryotic dichotomy, and adopting the outlook and value structure of molecular biology—delayed the establishment of a phylogenetic framework for microbiology for more than a decade, causing that discipline's stunted development, with the unfortunate consequences I mentioned. Even today, more than a decade after the discovery of the *Archaea*, many biologists *still* don't question the phylogenetic validity of the taxon "prokaryote"!

Can you understand why I have such distaste for the prokaryote-eukaryote dichotomy? This is not the unifying principle that we all once believed it to be. Quite the opposite: it is a wall, not a bridge. Biology has been divided more than united, confused more than enlightened, by it. This prokaryote-eukaryote dogma has closed our minds, retarded microbiology's development, and hindered progress in general. Biological thinking, teaching, experimentation, and funding have all been structured in a false and counterproductive dichotomous way. The little that is gained by knowing that the world of living systems comprises two distinct cytological types (very loosely defined in the case of prokaryotes, I might add) is far outweighed by the pernicious evolutionary implications this simplistic formulation holds—namely, that all prokaryotes share a common ancestry apart from that of eukaryotes; that archaea, therefore, are "just bacteria"; and that prokaryotes are simpler than, arose earlier than, and represent the ancestors of, the eukaryotes. Some of this may indeed turn out to be true, but these are matters for discussion and experimentation; they are not our catechism.

That is my view of the historical events that were principally responsible for the state of microbiology through the latter part of the 1970s. By no fault of their own, microbiologists had failed early on to determine the natural relationships among prokaryotes, despite their valiant efforts to do so (15, 26). Microbiology consequently developed without the evolutionary dimension essential to defining and understanding any biological system. Lacking this unifying framework, without a central core, without a real "concept of a bacterium," without a sense of itself, microbiology could not develop "ontogenetically," as it were, from its own internal roots. The discipline lacked cohesion; it was a collection of anecdotal facts given shape by external forces (practical considerations and its utility to biochemists and molecular biologists). Microbiology was a building supported (not to mention defined) by a scaffolding. Such a discipline became the weak sister of the biological sciences and, as such, easy prey for other disciplines that would grow at its expense. It is truly unfortunate that the early microbiologists were unable to determine the natural relationships; they saw the problem so clearly. By the time that they were finally presented with the techniques for doing so, microbiologists had lost sight of the value of the enterprise and so were blind to the power that had been laid before them. That was not only unfortunate. It was tragic.

### THE NATURAL ORDER OF THINGS

Today the natural relationships among bacteria can be, and are being, determined. Microbiology is finally a complete biological discipline, resting on a firm historical (evolutionary) base, drawn together by a unifying principle. A new, transformed microbiology must now take its rightful place among the biological sciences. Generations of training in the old ways have made us complacent about the state of our discipline,

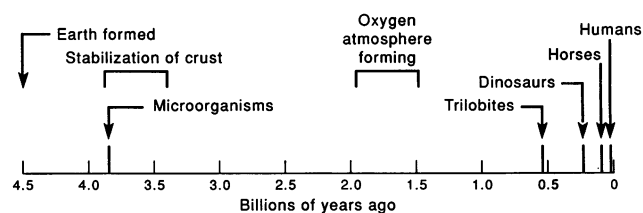


FIG. 2. Time line for the planet Earth. Various salient events in the planet's history are shown, including the times at which certain major evolutionary groups are thought, from fossil evidence, to have arisen.

however: "What do you mean, we need to change the way we do microbiology?" "Determining the natural relationships straightens out the mess microbial taxonomy got itself into; it doesn't change *microbiology*." "Microbiology never has and never will have anything to do with the study of eukaryotes, save in practical terms (medical and so on)." "Face it, microbial biochemistry is microbiology." Well—the first hurdle we have to get over is merely wiping the old slate clean! This time we are in a position to realize that the paradigm is shifting, to see what's happening. Only at that point will we feel in our bones that the natural relationships, the evolutionary trace, will infuse *all* aspects of microbiology, making them richer, deeper, more interconnected, and more valuable. And only then will we be in a position to effectively guide microbiology's journey into its New World.

We can make a good start at an understanding by focusing on the position prokaryotes hold in the natural order of things. Just think of the ways in which the microbial world surrounds, supports, delimits, exceeds, and generates the world of (multicellular) eukaryotes. Think, too, of how little these things are appreciated! A perfect example here is the wrong-headed image people—informed laymen, students, science writers, and professionals—have of what is "ancient" in an evolutionary sense. Figure 2, a time line showing when in this planet's history various interesting biological entities arose, well illustrates the point: the bacteria are nearly as old as the planet (20, 31). Those ancient and formidable Animal and Plant Kingdoms are recent divergences, near cousins, from this perspective. By this standard dinosaurs and trilobites are trivial, Lucy (*our* ancestor) almost laughable. Yet, for most of us, especially the young grade school and high school minds, it is precisely the dinosaurs and trilobites that epitomize "ancient" (not to mention powerful and mystical). What was it that the Harvard paleontologist Stephen Jay Gould recently wrote? (I could not believe my eyes!) "... we live in the Age of Bacteria (as it was in the beginning, is now and ever shall be, until the world ends). . ." (11). How well said!

Equally remarkable is the evolutionary debt that multicellular eukaryotes owe the prokaryotes. Our feeling for living things naturally begins with animals. That feeling quickly broadens to include plants, especially as we come to appreciate that without their ability to use the Sun's energy to produce biological substance, there would be no animal life. Yet, how is it that plants have this marvelous capacity to harness sunlight? As we biologists know, plant cells contain entities called chloroplasts, which have the capacity to photosynthesize. But chloroplasts did not evolve as a part of the plant cell; they were acquired by the (evolving) plant cell from the bacterial world, through endosymbiosis. In other words, it is the bacteria that have invented photosynthesis, and plant chloroplasts are merely domesticated cyanobacteria. The same, as you know, applies to the mitochondria, the organelles that allow (almost)



all eukaryotic cells to utilize oxygen as an energy source. Mitochondria too had bacterial endosymbionts as ancestors, in this case the endosymbionts came from the so-called purple bacterial group. Let's face it; without that marvelous bacterial invention, photosynthesis, there would be no plants—no giant sequoias, no endless fields of grain, no beautiful flower gardens. And there would be no animals without both plants (read cyanobacteria) and mitochondria. The next time you look under the microscope at photosynthetic bacteria, don't think of them as alien.

Being aware of our incredible genealogical debt to the microbial world, it makes my blood almost boil to see the prokaryotic cell humbled before its eukaryotic counterpart: "... the series of evolutionary steps in cellular organization leading from the prokaryotes to the eukaryotes [resulted] in an entirely novel level of organization [which] was surely the most drastic change in the whole history of the organic world" (16). To calm myself I step back and look up, and there I see the apostles of photosynthesis, Winogradsky and van Niel, standing, smiling—knowing and serene.

Prokaryotes underlie all living processes. Even the overall state of the biosphere is largely of their doing: our oxygen atmosphere exists (directly or indirectly) because of them. They are vital to the regulation of the planet's surface temperature, through their roles in carbon dioxide turnover and methane production (and utilization). Biological cycles turn on them—these ultimate recyclers. Have you ever seen the enormous banded iron deposits in the Lake Superior region (laid down roughly two billion years ago)? Do they suggest bacteria to you? They probably should!

Life on this planet can live under an incredible variety of conditions—prokaryotic life, that is. Some prokaryotes will grow below freezing, others above the (normal) boiling temperature of water. The members of the *Archaea* in particular are denizens of hot springs, and in some cases they drastically alter these environments: *Sulfolobus* species, for example, excrete a great deal of sulfuric acid, and so markedly decrease the pH of boiling mud pots. (Talk about learning to live with your pollution!) Deep-sea hydrothermal vents harbor prokaryotic communities. We, however, see TV specials about the *eukaryotic* communities there—spectacular tube worms, clams, crabs, and clouds of tiny shrimp. But without prokaryotes as the base of the food chain (and as symbiotic structures *within* these eukaryotes), these deep-sea "Disney Worlds" would not exist (23).

Extreme halophiles thrive under conditions that would pickle other things, i.e., in saturated brines. The deep border of the trench at the bottom of the Red Sea, hot, saline, and loaded with toxic heavy metal ions—is even a place where prokaryotes can make a living. And other heavy-metal-tolerant prokaryotes have been used in ore leaching. The soil: prokaryotes are *in* the soil, and in large measure *are* (they create) the soil (the fertile soils, that is). But their penetration of the planet doesn't stop there. We are beginning to find out that prokaryotes can grow deep underground, in oil deposits, and probably even in the ubiquitous cracks that run through rocks. The entire surface of this planet down to a depth of at least several kilometers may be a habitat for prokaryotes (10). It's prokaryotes all over, and prokaryotes all the way down! And to think that we try to cram them into a single department, not to mention, in many cases, a lackluster program within some larger department. Why, there should be departments of Cyanobacteriology, of Myxobacteriology, of Archaeology—and, of course, a department of Eukaryotes. Yet students today want to study more "relevant" things!

The biochemical diversity in the microbial world is stagger-

ing. Prokaryotes are the real chemists of this planet. Increasingly, their human counterparts turn to them (or to their enzymes) for subtle organic syntheses. A whole new industry based upon thermostable biocatalysts is in the offing.

## THE RENAISSANCE: MICROBIOLOGY'S FUTURE

Now it is time to be specific about the future of microbiology. Despite what you may think, I am not going to tell you that its future lies in studying microbial evolution. Yes, microbial evolution will be a fruitful area of research; it will even become the focus of Evolution departments. But the main effect microbial evolution will have on microbiology will be in providing the cohesive superstructure within which the discipline will operate.

Microbial ecology today indicates what the future holds. Here is a discipline in foment. Traditional microbial ecology was hamstrung by the need to cultivate microorganisms in order to give them (and their niches) meaningful characterization. The discipline relied heavily, as did all microbiology, upon enrichment culturing. The problem with this, as microbiologists all realized, was that not every species in a niche could be cultured and that enrichment culturing, by its very nature, could draw forth minor species, species that don't play significant roles in the niche. A less apparent but equally important limitation was in not knowing phylogenetic relationships. Without these, our picture of a niche is incomplete; it lacks richness, depth of understanding. For example, unless we know phylogenies, we can't say in many cases whether an organism in one niche is or is not identical to, similar to, or very different from some organism(s) in a different niche. What the microbial ecologist did in the past was, in effect, to create a zoo of monsters, laboratory freaks that best performed the (physiological) feats required of them. We could not know with certainty how this laboratory menagerie (and any understanding derived therefrom) related to the real world of microbial ecology.

It was Norman Pace who first comprehended the full extent of these limitations and how to overcome them. Microbial ecologists need to be able to take a truly representative census of any niche, at any time, as often as necessary. It is essential that they know the natural relationships among the organisms in any given niche, not to mention those between organisms in two different niches. This could all be done, Pace reasoned, by basing a census not upon cultured organisms but upon (rRNA) genes extracted and cloned *directly* from the niche itself. Sequencing a sufficient number of such clones would define what "phylotypes" occupy the niche and also determine their relative numbers. From these sequences, "phylogenetic stains" (specific probes) could then be designed, which would permit the organisms in a niche to be individually identified and counted (8, 23). The new institutes of microbial ecology being established around the world (which use these new methods) bear tribute to the salutary effect that microbial phylogeny in general, and Pace's insights in particular, are having on this discipline.

One of the more remarkable findings to emerge from the application of Pace's ecological approach is the discovery of what appear to be two unexpected new archaeal phenotypes (7). Although the organisms in question have yet to be cultured (or even identified with certainty under a microscope), their phylotypes are known. One of them is distantly related to the wall-less archaeon *Thermoplasma*; the other represents a deeply branching lineage among the *Crenarchaeota* (7). The habitat for these phenotypically uncharacterized archaea is the vastness of the central ocean, the largest of all biotopes on our

planet. In my judgement Pace's contribution to microbial ecology will rank historically with Beijerinck's development of enrichment culturing—to which it is a fitting complement.

Most real microbiologists (their numbers have dwindled in the last several decades) see the essence of the microbial world as lying in its diversity. Until now our understanding of microbial diversity was little more than a catalog of anecdotal facts, of which little or no overall sense could be made. This characterization, of course, applies to microbial biochemistry as well—which fact is seldom recognized because microbial biochemistry always produces interesting chemical complexity, and this alone is more than sufficient to fuel the never-ending search for new biochemical pathways and the like: Who cares whether they make any overall sense? Now the framework exists within which microbial diversity can be given connectedness, *can* make overall sense. The “big picture” that will almost certainly emerge here, in microbial biochemistry in particular, will give the study of microbial diversity a new significance and vitality. What an exciting prospect; the spectrum of diverse microbial biochemistries may be the framework within which we can effectively address the origin of life.

For me the most pleasing aspect of the “new microbiology” is the emerging connection between eukaryote and prokaryote. The discovery of the *Archaea*, i.e., finding that there exist two groups of prokaryotes no more related to one another than either is to the eukaryote, has been like adding a binocular component to previously monocular vision. With this finding the conceptual wall that had divided the study of the prokaryote from that of the eukaryote began to crumble; and the *rooting* of the universal tree (13), which reveals the archaea to be specific relatives of the eukaryotes, will bring that wall down forever. Studying the archaea becomes doubly exciting when you know that in so doing you may find a key to the evolution and nature of the eukaryotic cell.

The domain *Archaea* comprises a very disparate collection of (a small number of) phenotypes—methanogens, extreme halophiles, thermophilic sulfate reducers, extreme thermophiles (whose metabolism centers about sulfur and sulfur compounds), and perhaps a few others (see above)—which bear intriguing phylogenetic relationships to one another (14, 33) (Fig. 3). The distribution of phenotypes on the archaeal tree leaves little doubt that the domain *Archaea* is of thermophilic ancestry (33). Could this (phenotypically and ecologically) bizarre group, whose members all seemingly have relatively small genomes (somewhat less than two million base pairs on average) and are ancestrally thermophilic to boot, possibly hold the secret of the eukaryotic cell? The answer is, “Probably yes.”

Look at what is already known along these lines—and the surface has only been scratched. Two of the three eukaryotic RNA polymerases (II and III) find a homolog in the archaeal RNA polymerase; and both of the eukaryotic versions appear closer in sequence to their archaeal counterpart than they are to one another (18). The sequence of an archaeal histone (from *Methanothermobacter fervidus*) is reported to resemble eukaryotic histones H2a, H2b, H3, and H4—and, again, to be more similar to each of these than any of them are to one another (19). An archaeal heat shock protein structurally and functionally resembles eukaryotic chaperones, its sequence being most like that of the ubiquitous eukaryotic protein t-complex polypeptide-1 (28). And more pleasant surprises are on the way. At the University of Illinois Gary Olsen and I have been using a “sequence tag” strategy to explore the relationship of the *Archaea* to the *Eucarya*. By determining only 200 to 300 nucleotides from the ends of clones randomly selected from a library of *Thermococcus celer* DNA, it is possible to

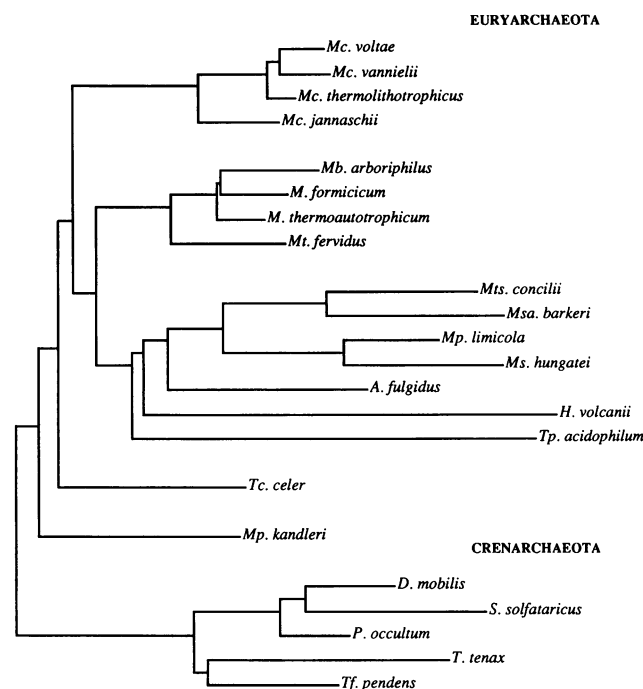


FIG. 3. Phylogenetic tree for (the culturable members of) the *Archaea*. The tree was produced from a transversion distance matrix for small subunit rRNA sequences (34a). The positions in the alignment used for the analysis met the condition that they were represented by a known nucleotide in all archaeal sequences used. Genus abbreviations in the various phenotypic groups are as follows: for methanogens, *Mc.*, *Methanococcus*; *M.*, *Methanobacterium*; *Mb.*, *Methanobrevibacter*; *Mt.*, *Methanothermobacter*; *Mts.*, *Methanosarcina*; *Msa.*, *Methanosarcina*; *Mp.*, *Methanoplanus*; *Mpy.*, *Methanopyrus*; and *Ms.*, *Methanospirillum*; for extreme halophiles, *H.*, *Haloferax*; for thermophilic sulfate reducers, *A.*, *Archaeoglobus*; for hyperthermophilic species, *D.*, *Desulfurococcus*; *S.*, *Sulfolobus*; *P.*, *Pyrodictum*; *T.*, *Thermoproteus*; *Tf.*, *Thermofilum*;  *Tp.*, *Thermoplasma*; and *Tc.*, *Thermococcus*.

search the data bases for the (eu)bacterial or eukaryotic homologs of these sequences. In about 10 to 15% of the cases the archaeal sequence readily turns up a relative in one or both of the other two domains. It is pleasing to see the number of cases in which the greatest resemblance, or even the only detectable homology, is with a eukaryotic gene, for example, the transcription initiation factors.

But how could an archaeal genome of less than two million base pairs tell us much about a eukaryotic cell that has a genome *many* times that? Wouldn't a small fraction only of eukaryotic genes find counterparts among the *Archaea* at best? Well, the dynamics of the evolutionary process may work to our advantage here. Eukaryotic genes tend to group into families, which share a common ancestor. And, from the little we know (see above), the sequences of the archaeal homologs of eukaryotic genes tend to most closely resemble the (inferred) sequences of the *ancestors* of the eukaryotic gene families. So, if the majority of eukaryotic genes can be grouped into large enough families, there is hope that much of the genome of the ancestral eukaryotic cell will compress (evolutionarily) into the genomes of the *Archaea*. The functional facet to this problem must be stressed: if an archaeal gene sequence resembles most closely the (inferred) sequence of the ancestor of a eukaryotic gene family, then the corresponding archaeal *function* should give us a handle on what the aboriginal eukaryotic function was. We are only about two million



base pairs away from knowing an awful lot more about (*Archaea* and) the ancestor of the eukaryotic cell than we do now. Sequence archaeal genomes!

So, we come to the end of this Stanier Memorial Lecture, with, hopefully, a new feeling for an old science, a big view of the world of the small. It is essential that microbiology be perceived and practiced in a way consistent with the natural order of things: microbes are the base for and sustain all other life on this planet. Let us reorganize all of biology around microbiology. Let us study microbial diversity as it ought to be studied—in a comparative context. Let us make ecologists aware of the real ecology: we need a census of the microbial species on this planet. Let us utilize the evolutionary dimension to its fullest. Let us sequence the genomes of those microorganisms that represent pivotal points in the evolutionary course. Let us make biology whole again.

I would close with a quote that says as succinctly as any I have ever read what microbiology really is:

... the way in which I approach microbiology ... can be concisely stated as the study of microbial ecology, i.e., of the relation between environmental conditions and the special forms of life corresponding to them. ... this is the most necessary and fruitful direction to guide us in organizing our knowledge of that part of nature which deals with the lowest limits of the organic world, and which constantly keeps before our minds the profound problem of the origin of life itself.

What I particularly like about this quote is that it is from Beijerinck (3), on the occasion of his being awarded the Leeuwenhoek Medal, microbiology's highest honor, by The Netherlands Royal Academy in 1905! This great microbiologist certainly understood the place of microorganisms in the natural order of things. We would do well to follow his lead once more.

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